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Viral Status of Infants of Hepatitis C virus Seropositive Pregnant Mothers

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Abstract: background: Egypt has the highest prevalence of hepatitis C virus infection nowadays. HCV is a significant cause of morbidity and mortality all over the world. Children acquire the disease mainly via vertical transmission route. **Aim of the study**: was to evaluate the viral status in infants of HCV seropositive pregnant mothers and to determine the factors contributing for HCV acquisition and suspected risk factors for vertical HCV transmission. **Methods**: This cross sectional study was conducted on 1000 pregnant women who came for delivery at the Obstetric and Gynecological department, Fayoum University Hospital. Structured questionnaire were used to obtain medical required data. They were tested for hepatitis marker (HCV antibody & HBsAg). Infants of HCV infected women were tested for HCV RNA using real time PCR test at age ranged from 2-18 months. Twenty one infants of them were tested for HCV antibody. **Results:** The prevalence of HCV antibody among pregnant women is 4.4%, 81.8 % of them had positive HCV RNA by PCR. Only 15 (1.5%) of participated women were found to be HBsAg positive. Risk factors for HCV infection are old age, rural residence, circumcision, previous dental procedure, previous CS and husband and or other family member with HCV. All infants of HCV infected women had normal liver enzymes, at time of sampling. All 28 infants tested negative for HCV RNA by PCR. HCV antibody done for 21infants was negative. **Conclusions**: the prevalence of HCV antibody among pregnant women was 4.4%. All infant tested negative for HCV RNA. Vertical transmission of HCV is not established as route of infection in our study.

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1. Introduction

Hepatitis C virus (HCV) is the most common blood-borne disease worldwide. The Centers for Disease Control and Prevention (CDC) assessed that 2.7 to 3.9 million inhabitance in the US live with chronic HCV, and 70 million persons have HCV worldwide according to the World Health Organization ***(CDC, 2016)***. The assessed rate of chronic disease in members younger than 15 years old is 2.1-5 million worldwide ***(Blach et al, 2017; Nwaohiri et al, 2018).*** Egypt has one of the most widespread rates for HCV disease worldwide ***(Kandeel et al, 2017).*** The prevalence in children is high, at 5.8%-6% as compared with 0.05%-0.36% in the USA and Europe ***(Barakat & El-Bashir, 2011; El-Shabrawi & Kamal, 2013).***

Vertical hepatitis C transmission is the main reason for infancy HCV infection via intra-uterine and intra-partum transmission (**Benova et al., 2014**) and the risk is higher in HCV-positive adult female with a high viraemia at their pregnancy ***(Cottrell et al., 2013)***. When the density of maternal HCV was 1 million particles per milliliter, the vertical transmission rate is 36% ***(Bortolotti et al., 2007)*** and HIV co-infection ***(Baroncelli et al., 2016).*** In addition, intravenous drug use, invasive procedures and female sex of fetus are some of other risk factors for transmission ***(Aebi-Popp et al, 2016; Garcia-Tejedor et al, 2015).***

Children infected through the vertical transmission path display spontaneous resolution in around 25% patients by the age of 3 years (Yeung et al, 2007). The development to chronic liver infection and the evaluation of Hepatocellular carcinoma (HCC) is uncommon in infants and adolescents, yet slow progression can occur and is related with high morbidity and mortality rates via untreated patients. Treating infants and adolescents is significant to avoid

development of liver infection and disease transmission ***(Karnsakul & Schwarz, 2019).***

2. Patients and Methods

This work was carried out through two steps:

The First step was a cross sectional study involving 1000 pregnant women attendant in the delivery ward of obstetrics & gynacology department in Fayoum University Hospital to identify the widespread of hepatitis C virus between pregnant female.

The second step of the study was a longitudinal study of the children of infected women to identify the rate of vertical transmission.

Time of study:

From May 2017 to June 2019.

Inclusion criteria:

All pregnant women in late 3rd trimester or coming for delivery.

Exclusion criteria:

1. Pregnant women who received treatment for hepatitis C infection.
2. Pregnant women on immunosuppressive therapy (which could interfere with immune response to HCV infection).

Pregnant women included in the study were subjected to:

1. Detailed history taking to identify the risk factors for HCV acquisition and suspected risk factors for vertical transmission including (Age, parity more than two, history of previous blood product transfusion, any dental procedures, history of home delivery, husband and other household members positive for HCV).

The questionnaire estimated socio-demographic criteria, present and past history, and potential risk factors for HCV acquisition and risk for vertical transmission.

2. Specimen collection and serological testing of pregnant women.

Blood samples were collected from the pregnant women and sent for assessment of hepatitis markers (anti- HCV antibodies& Hepatitis Bs Ag) and alanine transaminase level.

3. Mothers with positive anti – HCV antibody were subjected to HCV RNA testing by PCR to confirm the presence of HCV infection.

Infants of seropositive pregnant mothers included in the study were subjected to:

I. Follow-up at the Pediatric Hepatology clinic, Fayoum University Hospital and they were subjected to the following:

1. History taking: Infants were subjected to full history taking from the mother including: personal (including demographic data), perinatal history, History of any attack of (jaundice, abdominal distension, bleeding tendency) , past history (previous hospital admissions or Blood transfusion) and developmental history

2. Thorough clinical examination:

- Anthropometric measurements, vital signs
- Regional examination: Head and neck, extremities, skin and back
- Local examination: Cardiac, chest, abdominal and neurological examination.

II. These infants were tested for the presence of HCV infection by

1- Performing polymerase chain reaction test to detect HCV RNA.

2- HCV Antibody was done to 21 infants of HCV infected mothers.

At an age ranging from 2 months to 18 months. Serum transaminases (ALT & AST) also was done.

Laboratory investigations:

1-Sample collection from pregnant women

About 4 milliliters (ML) of venous blood were collected from each pregnant mother by venipuncture in to sterile plain plastic vacutainer. Samples were withdrawn using acceptable sterile medical techniques to avoid hemolysis.

The whole blood specimen in the plain vacutainers was allowed to clot completely at room temperature and then centrifuged immediately using tehtnica CENTRIC 322 Acentrifuge at 3000 rpm for 10 min to obtain serum. The separated clear and non hemolysed serum sample was sent for hepatitis markers (Anti HCV Ab & HBsAg) using chemillusence immunoassay by architect i 1000 SR and to determine ALT level using the fully automated biochemical analyzer (Beckmen coulter AU480).

2-Sample collection from infant of seropositive pregnant mothers

A sample of 5 ml of fresh venous blood was collected from peripheral vein by sterile venipuncture technique, and was divided into 2 sterile plain vacutainer. The separated clear and non hemolysed serum sample was sent to determine ALT&AST levels and to determine HCV RNA PCR using real time COBAS AmpliPrep/COBAS TaqMan HCV Test. Also, serum sample was sent for Anti HCV antibody using third generation ELISA test.

Statistical Analysis:

The sample size of 810 pregnant mothers was determined using Epi info 7 software. We assume that the expected prevalence of HCV was 5%, with a confidence interval of 95% and margin of error of 1.5%. Finally, 25% non-response rate was added to the calculated sample size to reach 1000 cases.

Statistical analysis was done using IBM SPSS, version 26 software for Windows. Univariate analysis is done as descriptive statistics for continuous variables showing Minimum, Maximum, Mean and

Standard Deviations and for categorical variables the frequency and percentages are reported .

Bivariate analysis is done to check for the association between different factors and the presence HCV infection in the mothers. To study the association between categorical variables and the presence of HCV infection, Chi-square test for independence is used and if the observed or expected values are less than 5 then Fisher's Exact test is applied. The association between numerical variables and the presence of HCV infection was tested using independent t test .

Multivariate analysis was done using multiple logistic regression comparing mothers with HCV to normal mothers. The aim of the logistic regression performed is to check for the association between the HCV infection and different factors while controlling for the others. Statistical significance was set at P< 0.05 for all analysis.

3. Results:

This cross sectional study was carried out from May 2017 to June 2019. It included 1000 pregnant women who came for delivery at the Obstetric and Gynecological department, Fayoum University Hospital, and were randomly selected and screened for HCV and HBV infection which became a routine measure performed in our hospital since 2016.

In search for HCV seroprevalence among the 1000 pregnant mothers 44 (4.4%) of them had HCV Ab positive and 36 of them (81.8%) had also positive HCV RNA by PCR.

The level of viremia of HCV was defined as follow:

Negative: when the viral load was below the detectable level <16 IU/ml

Low: viral load < 200000 IU/ml

Moderate: viral load 200000- 2000000IU/ml

High : viral load > 2000000 IU/ml.

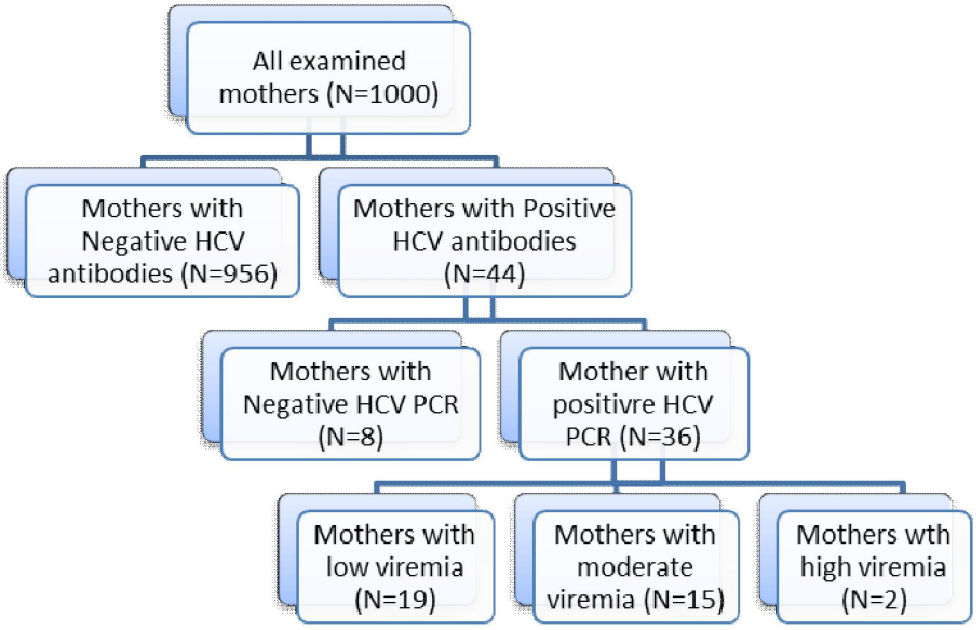

Figure (1): Schematic analysis of the studied pregnant mothers.

Transaminases (ALT, AST) were performed in only 153 mothers. The mean ± SD serum level of ALT was 20.29± 15.9 IU/mL and of AST was 23.95±11.419 IU/ml.

High ALT level was found in six mothers (3.9%). Among the six mothers with elevated ALT serum level, three of them (1.96%) were seropositivity for HCV and HCV RNA, 2 of them had moderate viremia (HCV RNA level 234000 and 294000 IU/ml) and one had low viremia (620 IU/ml). The other three pregnant women with elevated serum ALT level were HCV Ab negative and were negative for Hepatitis B virus markers and they had no other illnesses as diabetes mellitus or history of drug intake.

Table (1): Comparison of sociodemographic characters of both HCV AB +Ve and HCV Ab –ve groups.

variables		Hepatitis C virus infection status		P value
		HCV Ab positive (n= 44)	HCV Ab negative (n=956)	
Age group	< 20 years old	0 (0.0%)	59 (6.2%)	**0.025***
	20- 35 years old	35 (79.5%)	805 (84.2%)	
	>35 years old	9 (20.5%)	92 (9.6%)	
Maternal education	Not educated	13 (29.5%)	158(16.5%)	0.064
	Less than high school	5 (11.4%)	120 (12.6%)	
	High school	24 (54.5%)	538 (56.3%)	
	Some college or graduate	2 (4.5%)	140 (14.6%)	
Residence	Rural	40 (90.9%)	720 (75.3%)	**0.018***
	Urban	4 (9.1%)	236 (24.7%)	
Occupation	Health related	0 (0.0%)	31 (3.2%)	0.113
	Not health related	0 (0.0%)	60(6.3%)	
	House wife	44 (100%)	865 (90.5%)	

Chi square test and Fisher's exact test *Significant P value < 0.05

Analysis of the factors that could be associated with HCV infection showed a statistically significant association between HCV infection and different age groups, a higher percentage of HCV positive women were above 35 years (20.5%) compared to 9.6% in the HCV negative women (P-value = 0.025) Also. residence showed a statistically significant association with HCV infection, as a higher percentage of HCV positive women were from rural areas 90.9% compared to 75.3% of HCV negative women (p value=0.018).

Table (2): Association between HCV infection and possible risk factors for HCV acquisition.

Variables	Hepatitis C virus infection status		P value
	HCV Ab positive (n= 44)	HCV Ab negative (n=956)	
Previous hospitalization	1 (2.3%)	29 (3.0%)	0.772
Blood product transfusion	11 (25.0%)	166 (17.4%)	0.194
Previous abortion	17 (38.6%)	258 (27.0%)	0.091
Previous operation	9 (20.5%)	292 (30.5%)	0.154
Previous CS	36 (81.8%)	645 (67.5%)	**0.046***
Major accident	1 (2.3%)	6 (0.6%)	0.271
Circumcision	44 (100.0%)	801 (83.8%)	**0.004***
Dental care	26 (59.1%)	324 (33.9%)	**0.001***
Home delivery	9 (20.5%)	196 (20.5%)	0.994
Injection drug use	0 (0.0%)	2 (0.2%)	1.000
Needle sticks	0 (0.0%)	21 (2.2%)	0.620
Hijama	0 (0.0%)	5 (0.5%)	1.000
Schistomiasis history	0 (0.0%)	2 (0.2%)	1.000
HCV positive husband	10 (22.7%)	74 (7.7%)	**0.004***
Family member with HCV	23 (52.3%)	289 (30.2%)	**0.006***

Chi square test and Fisher's exact test *Significant P value < 0.05

Comparison of the risk factors that could be associated with HCV infection in both HCV +Ve Ab and HCV –ve Ab showed that the anti HCV positive women were more likely to be exposed to circumcision (p value= 0.004), had a history of dental procedure (p-value = 0.001) and had previous CS (P-value = 0.046).

Also, the frequency of having an HCV positive husband was 22.7% in HCV positive women which was higher than 7.7% in HCV negative women (p-value = 0.004). In addition having a family member with HCV was 52.3% in HCV positive women compared to30.2% in HCV negative women (p-value = 0.006).

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Table (3): Factors associated with increased risk of HCV infection.

	Odds Ratio	P value	95% C.I for OR	
			lower	upper
Maternal education				
Less than high school	0.763	0.633	0.252	2.314
High school	0.929	0.852	0.429	2.012
Some colleague or graduate	0.376	0.221	0.078	1.800
Rural residence vs. urban	2.406	0.108	0.825	7.016
Blood product transfusion	1.315	0.471	0.625	2.763
Previous abortion	1.258	0.496	0.650	2.435
Previous procedure	0.506	0.085	0.234	1.098
Previous CS	1.906	0.118	0.848	4.284
Previous dental procedure	**2.289**	**0.014**	**1.186**	**4.416**
Husband Positive for HCV vs. negative	**2.980**	**0.007**	**1.357**	**6.546**
Family member Positive for HCV vs. negative	**2.476**	**0.005**	**1.306**	**4.693**
No associated HBV	0.362	0.206	0.075	1.749

Logistic regression for factors associated with HCV infection was done for selected variables showed that the folds of increased risk of infection among pregnant mothers was associated with dental procedures OR 2.3 (95%CI: 1.2, 4.4) so the odds of HCV infection for those who had dental procedures is 2.3 times the odds of those who didn't have one.

Pregnant women who had HCV positive family member [OR 2.476- 95% CI (1.306-4.693)] had about two folds risk to have HCV, also those with HCV positive husband had about three folds risk [OR 2.980- 95% CI (1.357-6.546)].

Table (4): Socio-demographic characteristics of HCV Ab positive mothers (N=44).

variables	Number (N= 44)	%
Age (Mean age ± SD 29.32 ± 6.068 years old)		
< 20 years old	0	0.0%
20- 35 years old	35	79.5%
>35 years old	9	20.5%
Rural residence	40	90.9%
Working	0	0.0%
Education	31	70.4%
Positive family member with HCV	23	52.3%

The mean age of HCV seropositive pregnant mothers was 29.32 ± 6.068 years, with the majority (79.5%) belonged to 20-35 years group. More than 90% were from rural areas, about 70.4% were educated and more than half of them had a family member with HCV (52.3%).

The second Part of the study involved the neonates delivered to positive anti HCV Ab women, who were examined and followed at pediatric hepatology outpatient clinic, Fayoum University Pediatric Hospital.

Out of the 44 infants born to seropositive pregnant women, only **28(63.6%) infants** were enrolled in the study. Eleven infants (25%) were lost to follow up, 8 of them (18.2%) whose mothers were HCV RNA negative by PCR. There were 4 (9.1%) intrauterine fetal death (IUFD) and one case of stillbirth (2.3%)

The age of mothers of these cases of IUFD and stillbirth ranged from 25-35 years old, the mean ± SD gestational age was 35 ± 4.58 weeks ranged from 30-39 weeks, and all of them were multiparous except one was primigravida.

Table (5): Socio-demographic characteristics of participated infants at birth (N=28)

	Mean	SD
Gestational age (weeks)	38.1	1.4
	N	%
Gestational age		
<35 preterm	1	3.6%
36-37 near term	4	14.3%
>37 fullterm	23	82.1%
Sex		
Male	14	50%
Female	14	50%
PROM		
Yes	5	17.9%
No	23	82.1%
Mode of delivery		
CS	23	82.1%
NVD	5	17.9%

From the IUFD & stillbirth cases, two mothers had associated hepatitis B virus infection. All of them had moderate viral load level. All mothers were delivered by CS and all of them were exposed to circumcision, had a family member with HCV infection.

At birth, we found that the gestational age at birth of studied participant neonates ranged between 33 and 40 weeks with a mean gestational age 38.1±1.4 weeks. Both sexes were equally present in our study. Regarding the mode of delivery, 17.9% were normal vaginal deliveries; the history of PROM was present in 17.9 % of them.

Table (6): Perinatal history of participated infants.

	Mean	SD
Birth weight (kg)	3.0	0.5
	N	%
Birth weight		
Normal birth weight	28	100%
Low birth weight	0	0%
NICU Admission		
Yes	6	21.4%
No	22	78.6%
neonatal jaundice		
Yes	5	17.9%
No	23	82.1%

Regarding the birth weight, all cases were appropriate for GA at birth. Six infants (21.4%) had history of NICU admission, two of them admitted due to respiratory distress. There is history of neonatal jaundice in five cases (17.9%), four of these cases needed NICU admission for photo therapy. Otherwise, systemic examination was normal and revealed no abnormality.

Breast feeding was initiated in all cases except only one whose infant was admitted in NICU and she received treatment for HCV with oral DAA one month after delivery. Ten mothers completely weaned their infants to receive treatment at infant ages ranging from 5 months to 14 months, while others did not receive treatment till end of the study.

The majority of mothers did not have any illness during pregnancy, except one had gestational diabetes.

HCV PCR for infants was done at **an age ranging between 3 months to 18 months (≤18 months),** 5 infants (17.9%) were below 6 months , eleven (39.3%) were 7-12 months old and twelve infants (42.9%) were 13-18 months of age.

All 28 infants tested negative for HCV RNA by PCR. In addition, transaminases (ALT, AST) were performed for all infants. The mean ± SD serum level of ALT was 16.8± 8.2 IU/L and of AST 29.9± 9.1 IU/L. All 28 infants had normal liver enzymes.

Table (7): Age at HCV PCR sampling of infants (N=28).

	Mean	SD
Age at sampling (months)	12.1	5.4
	N	%
Age at sampling (months)		
≤ 6 months	5	17.9%
7-12 months	11	39.3%
13- 18 months	12	42.9%

Analysis of HCV Ab was performed for 21 infants. 2 of them (9.5%) were below 6 months, ten (47.6%) were 7- 12 months old and nine (42.9%) were 13-18 months of age and revealed that all of them were negative for HCV Ab. HCV Ab was repeated for the two cases that were below six months after 4 months and the result were negative again.

Table (8): Anthropometric measurements of the studied neonates (N=28).

	Mean	SD
Weight (kilogram)	9	2.1
Recumbent length	72.5	7.1
Head circumference	44.3	2.3
	N	%
Weight		
Below 3rd percentile	0	0.0%
Normal percentile	27	96.4%
> 90th percentile	1	3.6%
Recumbent length		
On 3rd percentile	1	3.6%
Normal percentile	27	96.4%
> 90th percentile	0	0.0%
Head circumference(HC)		
Microcephaly	0	0.0%
Normal HC	28	100.0%
Macrocephaly	0	0.0%

Table (8) reveals the anthropometric measurements of our studied infants. All cases had normal head circumference. Regarding body weight, only one case was overweight for age at time of examination. one infant had recumbent length on 3rd percentile.

Table (9): Developmental history of participated infants.

	N	%
Developmental history		
Normal	26	92.9%
Delayed motor development	2	7.1%

Examination of participated infants at time of sampling showed that all infants had normal motor and mental development, except for two cases who showed rackitic manifestation had delay in motor development which showed improvment following treatment.

Table (10): General examination of participated infants at sampling time (N=28).

	Mean	**SD**
Respiratory rate	30.5	4.8
Heart rate	108.5	9.3
	N	%
Pallor	12	42.9%
Jaundice	0	0.0%
Rickets	2	7.1%

All infants had normal vital signs with mean heart rate 108.5± 9.3 b/min and mean respiratory rate 30.5±4.8 breath / min. pallor was found in 42.9% and two cases only had ricketic manifestations.

Table (11): Systemic examination of studied infants (N=28).

	N	%
Cardiac		
normal	28	100.0%
abnormal	0	0.0%
Chest		
normal	26	92.9%
abnormal	2	7.1%
Abdominal		
no organomegaly	25	89.3%
hepatosplenomegaly	2	7.1%
splenomegaly	1	3.6%

Examination of different systems of the studied infants showed that cardiac examination was normal in all 28 infant with normal heart sounds and no murmur was heared. Two cases only had abnormal chest finding, harsh vesicular breathing and wheezy chest associated with rhinorrhea and fever, one of them needed admission and diagnosed as pneumonia. The majority of cases (89.3%) had no organomegaly. Two cases had mild hepatosplenomegaly at time of examination and only one had splenomegaly. Infant with organomegaly also had pallor.

4. Discussion

Viral hepatitis was evaluated to be the 7th major reason of mortality rate worldwide. Hepatitis C virus (HCV), an essential cause for liver fibrosis, cirrhosis and tumors is responsible for about half of mortality rate mentioned above ***(Hanafiah et al., 2013; Lavanchy, 2011)***.

The newly development of highly effective oral direct-acting antivirals (DAAs) provides opportunities for decreasing HCV infection burden and its onward transmission, with the potential for eradicating this blood-borne virus as a popular health problem ***(WHO, 2015; Ayoub & Abu-Raddad, 2016; CDC, 2015).***

Egypt is one of the countries most affected by hepatitis C virus. The Egypt Demographic and Health Surveys (EDHS) recorded antibody prevalence through the adult peoples aged 15–59 years at 14.7% ***(El-Zanaty & Way, 2009)*** in 2009 and at 10.0% ***(Ministry of Health and Population, El-Zanaty and Associates & ICF International, 2015)*** in 2015, substantially higher than global levels ***(Hanafiah et al., 2013; Lavanchy, 2011; Cornberg et al., 2011)***. To meet this challenge, Egypt has developed a national plan for hepatitis C prevention and established programs to eliminate and remedy viral hepatitis (Esmat, 2014). After effective negotiations to obtain 99% discounted rates for DAA prices ***(Kim et al., 2015)***, Egypt started an ambitious national HCV treatment program aiming to treat over 250,000 chronically infected persons per year, with the target of achieving a national chronic infection prevalence of <2% by 2025 ***(Ayoub & Abu-Raddad, 2016; McNeil & Donald, 2015)***. Despite this progress, existing evidence suggests ongoing HCV transmission in Egypt, with higher incidence levels relative to other countries ***(Ayoub & Abu-Raddad, 2016; Kouyoumjian et al., 2018).***

Egypt planned to survey 45 million inhabitants in one year beginning 1 October 2018, with regard to the World Health Organization core testing rules of providing approval, privacy, counseling, accurate results and connection to treatment for all individual who will be discovered positive. The purpose is to remedy all diagnosed patients from the surveying ***(Doss et al., 2018).***

Vertical transmission of HCV has gained importance as the primary route of HCV transmission among children especially following implementation of blood and blood product screening therefore assessment of the burden of vertical

transmission in countries with high HCV prevalence such as Egypt is essential ***(Benova et al., 2014).***

The purpose of this study was to evaluate the viral status in infants of HCV seropositive pregnant mothers and to determine the factors contributing for HCV acquisition and suspected risk factors for vertical HCV transmission.

This cross sectional study included 1000 pregnant women who came for delivery at the Obstetric and Gynecological department, Fayoum University Hospital.

The seroprevalence of HCV Ab among the pregnant women in the present study is reported to be 4.4 % and 81.8% of them had also positive HCV RNA by PCR which is similar to what was reported by ***Edessay et al. (2016)*** that prevalence of HCV Ab among mothers was 5.3% and HCV PCR was positive in 3.4%. Our findings are lower than previously recorded in similar researches. It was recorded to be 15.8% ***(Stoszek et al., 2006)***, 15.7% (***shebl et al., 2009)***, 11.7% ***(Zahran et al., 2010)***, 8.6% ***(Abdel-Qawi et al., 2010)*** and 6.1% ***(Khamis et al., 2014).*** This may reflect a better control policy with additional frequency reduction over time.

In our study, the prevalence of HCV infection during pregnancy in Fayoum which is one of the governorates of North Upper Egypt was 4.4% positive HCV Ab and 3.6% positive HCV PCR, which is very low in comparison to Delta governorates (10.4% positive HCV Ab and 8.2 % positive HCV PCR) ***(Edessay et al., 2015)***, also a study done by ***shebl et al. (2009)*** reported that 15.7% and 10.9% of pregnant women had HCV antibodies and HCV RNA, respectively. This may be explained by the prevalence of schistosoma infection in Delta Egypt is higher than in Upper Egypt which is constantly associated with HCV infection.

Although there is a declining trend observed as the prevalence of HCV among pregnant women in Egypt shows to have lowered, our prevalence of 4.4% is still greater than in other developing countries such as Pakistan (1.42%) ***(Ahmad, 2016)***, India (2.8%) ***(Goyal et al., 2014)*** and Nigeria (0.4%) ***(Clement et al., 2010).***

The prevalence of HCV Ab among pregnant women in Canada was 0.5% ***(McDermott et al., 2010).*** In Central Brazil, out of 28,561 pregnant females the widespread of HCV disease was 0.15% ***(Costa et al., 2009).***

The current work appeared that one of the risk factors for HCV infection between pregnant women was older age. In agreement with our results, ***Khamis et al. (2014), Edessay et al. (2016), Abdel-Qawi et al. (2010) and Costa et al. (2009)*** reported the relationship among HCV disease and older age of the pregnant females. Contrary to the current study data, Zahran et al. (2010) appeared that there were no considerable variations in mean age of the HCV seropositive and seronegative pregnant females.

The greater rate of disease between the older age can be elucidated by the cumulative influence of exposure to HCV caused by prolonged exposure to the virus over an individual's lifespan, as well as exposure to another potential HCV risk factors

In the present work, spread of anti-HCV was greater between pregnant women residing in rural versus urban regions (90.9 % vs. 9.1%). As reported by ***El-Gharably et al. (2017)***, higher infection rates in rural areas may be explained by the major reservoir of chronic HCV disease established in the course of PAT campaigns and related concurrent infecion control measures remains the most likely cause for the high spread of HCV, which may be largely responsible for the continuing disease transmission of HCV in Egypt today. Other methods of transmission such as low infection control and equipment sterilization methods applied in rural medical and dental tools also contribute to ongoing iatrogenic HCV diseases and continue to fuel spread of infection

Female Circumcision was found to be one of HCV infection risk factors in the present study. This may be due to female circumcision practice done at home by non-medical personnel with unsterilized instrument and this may be responsible for persistence and propagation of infection especially in rural areas. This was also reported in other studies ***Ali et al. (2017) and kamal & collegue (2008).***

In this study, other risk factors associated with HCV transmission were previous CS, dental procedures, HCV positive husband and other family members with HCV.

In the current study, history of dental procedures was more prevalent among seropositive women. Many other works appeared that there is an evidence of ongoing HCV occurrence at dental and medical equipment's ***(Kalil et al., 2010; Barakat & El-Bashir, 2011).*** Although most researches have not found dental methods to be a risk for HCV in Egypt or elsewhere ***(Habib et al., 2001; Enomoto et al., 2001).***

In our study, history of caesarean section was a significant risk factor for HCV infection. This may be explained by the low socio-economic status of most rural residents who search for the lowest cost in medical services, where delivery may not be done in well-equipped hospital with proper infection control.

Other risk factors for HCV acquisition among pregnant women participating in this study were having a husband or another family member positive for HCV found in both univariate and multivariate

analysis. This association suggests the role of intra-familial transmission of HCV and role of sexual transmission. Our finding is similar to previous studies that showed intra-familial role of HCV transmission ***(Omar et al., 2017; El-Dien et al., 2014; Lankarani et al., 2016).***

Transmission between sexual partners of persons with chronic HCV infection with no other risk factors for infection is about 5 % (range, 0–15 %) ***(Awan et al., 2006; Faridulla et al., 2002).*** However, marriage did not only involve a sexual relationship but also different practices of body contact and subjected to the same risk factors (ie, using the same personal tools, subjected to blood of the index case by any means) ***(Cavalheiro et al., 2009).***

In our study, history of blood transfusion was found in (11/44) of HCV seropositive mothers and 1.1% of all screened women compared to 16.6% of seronegative women. On the other hand, Zahran et al. (2010) showed a history of blood transfusion in 6.6% of HCV antibody positive versus 2.2% in those who were negative. AbdulQawiet al. (2010) also showed blood transfusion as a risk factor for disease (15% of HCV antibody positive females had transfusion versus 3.9% between HCV antibody negative females). This can be explained by effective blood product screening in Egypt nowadays.

In our study, HCV PCR was performed to 28 infants of the 44 seropositive mothers, and 82% of them were tested at an age of or older than 7 months. ***Chappell and colleagues (2018)*** reported that the best screening for perinatal HCV transmission was described as HCV screening test as 1) HCV RNA by Polymerase Chain Reaction (PCR) prior 18 months of age or 2) HCV Ab check following 18 months of birth.

All 28 infants in our study performed HCV RNA by PCR tested negative which was done in the first 18 months of life. Also, HCV Antibody was performed for 21 infants, 90% of them at 7- 18 months of age and all of them had negative HCV Ab. ***Epstein and co-workers (2018)*** predicted a negative anti HCV by 12 months in 70% of infants and in 99% by 18 months of age. ***Chappell et al. (2018)*** stated that infants who had a negative HCV antibody screening test result at any time or 2 negative HCV RNA test results were intended to be negative for HCV disease. To confirm the negative HCV RNA by PCR we tried to contact the mothers to repeat the test but all of them refused as first test were negative and their infants were growing well without any symptoms.

In contrast, there were several studies that showed a higher vertical transmission rate in Egypt, 11.2% of infants of HCV infected mother had positive HCV PCR immediately after birth from cord blood sample ***(Ali et al., 2017)***, 17.39% of infants were positive for HCV Ab and HCV RNA in the first 24 h post-delivery ***(Abd Elrazek et al., 2016),*** 13% of infant's HCV PCR was positive at first month of life and only 3.8% of infant's HCV PCR was positive at 6months of age ***(Abdel-Qawi et al., 2010)***. ***Shebl et al. (2009)*** reported that 10% of infants tested positive for HCV Ab and HCV RNA 2 months following birth. Of them, 4.6% remained positive for HCV PCR at one year of age and 2.4% had positive HCV PCR at 2-3 years of age.

Many researches have estimated the risk of HCV vertical transmission with conflicting data. Indeed, the rates of transmission ranged from 0% to 30% ***(Tosone et al., 2014)***. These great variation are probably due to differences in trial sample (e.g., the number of HCV-infected mothers enrolled), the study procedures (prospective or retrospective study; detection of maternal infection established on anti-HCV antibody positivity or on HCV RNA positivity) and the diagnostic characteristics of neonatal HCV infection e.g., number of polymerase chain reactions proceeded and period and timing of follow-up in the neonates ***(Tosone et al., 2014).***

Researches that do not test children as they grow may lead to an overrate of HCV spread and this may be the case with early community investigations of perinatal transmission of HCV in 3 rural Egyptian villages, where HCV is generally more prevalent of 20% (Stoszek et al., 2006; Saleh et al., 2008). Frequent clearances of perinatal HBV infection, despite previous reports of a high incidence of vertical transmission in Egypt, may be distorted by the fact that these records rely on cord blood samples or PCR outcomes only once in within a few weeks after birth ***(Abdel-Qawi et al., 2010).***

Vertical transmission rates range from 5% to 6% among HCV-mono-infected women to 10% among those with HIV co-infection ***(Chappell et al., 2018)***. ***Arseniki & Christina (2018)*** stated that HIV Co-infection is a well-established risk factor of HCV vertical transmission, with transmission rates 2.82 fold higher than HIV-negative mothers. Also, high viral load of HCV in pregnant mothers is associated with increased risk of vertical transmission. This may explain our findings as HIV is very rare in Egypt and only 2 pregnant women had high HCV viremia, one of them was lost to follow-up.

The leading cause for the low vertical transmission rate has not been identified. Several biological and immunological parameters can preserve the embryo against HCV disease, such as placental immune cells, embryo adaptive cellular immunity, embryo plasma inflammatory markers,

maternal HLAII alleles, and the IL-28B genotype. ***(Tosone et al., 2014; Le Campion et al., 2012).***

In our study, among 44 infants of HCV seropositive mothers, there were 4 (9.1%) intrauterine fetal death and one case of stillbirth (2.3%). Two of their mothers had hepatitis B virus co-infection. All of the 5 mothers had moderate HCV RNA level in their blood. Obstetric causes of intrauterine fetal deaths were not known.

Among the 28 infants enrolled in the study, only one (3.6%) was born preterm at 33 weeks gestation, 14.3% was born near term (36-37 weeks). All of them were appropriate for gestational age. Six infants were admitted in NICU, two of them admitted due to respiratory distress and one of them required assisted ventilation. Only one seropositive HCV mother had gestational diabetes.

Pergam et al. (2008) comparing 506 HCV-positive pregnant females with 2022 HCV-negative pregnant controls. In multivariable analysis, it was found that children born to females infected with HCV were more probable to be small for gestational age, have low birth weight, need admission to the neonatal intensive care unit, and need assisted ventilation. One more population-based retrospective cohort study based in Florida by Connell et al. (2011) compared 988 HCV-positive females with 1,669,370 controls. Using multivariate examination, it was recorded that HCV-infected females were more likely to birth children with poor birth outcomes, involving early delivery, low birth weight, and congenital anomalies. A meta- analysis that involved these two tests and five others reported that maternal HCV infection was significantly related with embryo growth restriction and low birth weight ***(Huang et al., 2016). Reddick et al., (2011)*** documented greater rates of gestational diabetes in HCV-infected females in comparison to non- infected females.

Limitation

Limitation of our study is the inability to confirm the negative HCV RNA in all infants aged below 18 months for the second time. Also, the eight mother-infant pairs who were lost to follow-up while the mothers had a negative HCV RNA that needed also to be confirmed. In addition, all infants should be followed till the age of 36 months to rule out perinatal HCV transmission according to the CDC guidelines.

Conclusions:

The prevalence of HCV antibody among pregnant women is 4.4%, 81.8% of them had positive HCV RNA by PCR. All HCV infected women were newly diagnosed and did not know their HCV status before screening during pregnancy.

Our results suggested that there is an association among old age and rural residence of the tested females and HCV acquisition. Other risk factors for HCV acquisition were: circumcision, previous dental treatment, previous caesarean section and husband and/or other family member with HCV infection .

All infants of HCV infected women had normal liver enzymes, at time of sampling. All 28 infants tested negative for HCV RNA by PCR which was done at an age ranging from 2-18 months. HCV antibody done for 21infants was negative .

Recommendations

- Pregnancy offers a good chance to screen for viral hepatitis and link women who may not otherwise seek medical advice especially, those in the childbearing period who are a considerable part of the community.
- Implementation of national screening program for HCV in pre-marital females is recommended for the necessity to start the highly efficacious oral DAA treatment prior to marriage, which in turn can eliminate HCV vertical transmission.
- HCV-infected pregnant women should receive adequate counseling by obstetric and primary care providers regarding the risk of perinatal HCV transmission and the need for postnatal HCV screening.
- Mass campaign to raise public awareness about viral hepatitis and the modes of transmission especially in rural areas, where traditional risky behavior as circumcision still prevailed, should be offered.
- Preventive measures for HCV infection especially adequate sterilization of surgical and dental instruments should be used.
- Integration of HCV infected mother – infant into a system of scheduled visits such as the vaccination schedule, which link them to medical care in order to reinforce the proper timing of infants screening and avoid the follow-up lost visits.

Conflict of interest

All authors declare that they have no conflict of interest.

References

[1]. ***Abd-Elrazek, A., Amer, M., El-Hawary, B., Salah, A., Bhagavathula, A. S., Alboraie, M., & Saab, S.*** Prediction of HCV vertical

transmission: what factors should be optimized using data mining computational analysis. Liver International 2016; 37(4), 529–533.

[2]. ***AbdulQawi K, Youssef A, Metwally MA, Ragih I, AbdulHamid M, Shaheen A.*** Prospective study of prevalence and risk factors for hepatitis C in pregnant Egyptian women and its transmission to their infants. Croat Med J. 2010; 51(3):219–228.

[3]. ***Ahmad I.*** Prevalence of Hepatitis B and C Viral Infection Among Pregnant Women in Peshawar, Pakistan. Hepat Mon. 2016; 16(6):e36383.

[4]. ***Ali Abd El-Naser***. ….et al. Prevalence of hepatitis C virus among pregnant women of Upper Egypt. European journal of pharmaceutical and medical research Ejpmr. 2017; 4(2),76-81

[5]. ***Arsenaki E, Christina B***. An Overview of Risk Factors of Vertical Transmission of Hepatitis C Virus and Treatment Options for Chronic HCV. J Virol Antivir Res. 2018; 7:2.

[6]. ***Arshad M, El-Kamary SS, Jhaveri R.*** Hepatitis C virus infection during pregnancy and the newborn period. Are they opportunities for treatment? J Viral Hepat 2011; 18: 229-36

[7]. ***Awan NS, Asraf N, Nayyar S.*** Obstetrics and perinatal outcome; risk factors for hepatitis B and C transmission. Professional Medical J. 2006; 13(4):511–516.

[8]. ***Ayoub, H. H. & Abu-Raddad, L. J***. Impact of treatment on hepatitis C virus transmission and incidence in Egypt: A case for treatment as prevention. Journal of viral hepatitis 2016; 31, 12671.

[9]. ***Barakat SH and El-Bashir N***: Hepatitis C virus infection among healthy Egyptian children: prevalence and risk factors. Journal Viral Hepat. 2011; 18(11): 779-784.

[10]. ***Baroncelli S, Pirillo MF, Amici R, et al.*** HCV-HIV co-infected pregnant women: data from a multicenter study in Italy. Infection 2016; 44: 235–42.

[11]. ***Benova L, Mohamoud YA, Calvert C, Abu-Raddad LJ.*** Vertical transmission of hepatitis C virus: systematic review and meta-analysis. Clin Infect Dis. 2014; 59(6):765-773.

[12]. ***Blach S, Zeuzem S, Manns M, et al.*** Global prevalence and genotype distribution of hepatitis C virus infection in 2015: a modelling study. Lancet Gastroenterol Hepatol. 2017; 2(3):161-176.

[13]. ***Bortolotti, F.; Verucchi, G.; Cammà, C.; Cabibbo, G.; Zancan, L.; Indolfi, G.; .. et al.*** Long-term course of chronic hepatitis C in children: From viral clearance to end-stage liver disease. Gastroenterology 2008, 134, 1900–1907.

[14]. ***Cavalheiro Nde P, De La Rosa A, Elagin S, Tengan FM, Araújo ES, Barone AA.*** Hepatitis C: sexual or intra-familial transmission? Epidemiological and phylogenetic analysis of hepatitis C virus in 24 infected couples. Rev Soc Bras Med Trop. 2009; 42(3):239–244.

[15]. ***Centers for Disease Control and Prevention.*** Hepatitis C. http://wwwnc.cdc.gov/travel/yellowbook/2014/chapter-3-infectiousdiseases-related-to-travel/hepatitis-c Published July 10, 2015. Accessed on April 24, 2017.

[16]. ***Centers for Disease Control.*** The ABCs of Hepatitis - for Health Professionals. 2016:3

[17]. ***Chappell CA, Hillier SL, Crowe D, Meyn LA, Bogen DL, Krans EE.*** Hepatitis C Virus Screening Among Children Exposed During Pregnancy. Pediatrics. 2018; 141(6):e20173273.

[18]. ***Clement, MI, Andy, EI, Eni, LO, Jewell, PA.*** Prevalence, sociodemographic characterestics and risk factors for hepatitis C infection among pregnant women in Calabar municipality, Nigeria. Hepat Mon 2010; 10: 116–120.

[19]. ***Connell, L.E.; Salihu, H.M.; Salemi, J.L.; August, E.M.; Weldeselasse, H.; Mbah, A.K.*** Maternal hepatitis B and hepatitis C carrier status and perinatal outcomes. Liver Int. 2011, 31, 1163–1170.

[20]. ***Cornberg, M. et al.*** A systematic review of hepatitis C virus epidemiology in Europe, Canada and Israel. Liver international: official journal of the International Association for the Study of the Liver 2011; 31(Suppl 2), 30–60, 1478-3231.

[21]. ***Costa, ZB, Machado, GC, Avelino, MM, Gomes Filho, C, Macedo Filho, JV, Minuzzi, AL.*** Prevalence and risk factors for Hepatitis C and HIV-1 infections among women in Central Brazil. BMC Infect Dis 2009; 9: 116–116.

[22]. ***Cottrell E, Chou R, Wasson N, Rahman B, Guise J-M.*** Reducing risk for mother-to-infant transmission of hepatitis C virus:a systematic review for the U.S. Preventive

Services Task Force. Ann Intern Med. 2013; 158(2):109-113.

[23]. ***Doss W; Jabbour J; Atta H; Hermez J; Hashish A.*** Towards a hepatitis-free Egypt: is this achievable? East Mediterr Health J. 2018; 24(7):609–610.

[24]. ***Edessy M.*** Hepatitis C Virus Infection during Pregnancy in Upper Egypt. International Journal of Current Research in Medical Sciences 2016; ISSN: 2454-5716 www.ijcrims.com Volume 2, Issue 10 -2016

[25]. ***El-Dien Nouh MA, AlFateh Mahrous OA, Zekry Farg IA.*** Intra-familial transmission of hepatitis C virus in Menoufia Governorate. Menoufia Med J. 2014; 27(2):478–483.

[26]. ***El-Gharably, A.I. Gomaa, M.M. Crossey, P.J. Norsworthy, I. Waked, S.D. Taylor-Robinson***. Hepatitis C in Egypt–past, present, and future. Int. J. Gen. Med. 2017; 10, p. 1.

[27]. ***El-Shabrawi MH, Kamal NM***. Burden of pediatric hepatitis C. World J Gastroenterol. 2013;19(44):7880-7888.

[28]. ***El-Shabrawi, M. H. F., Kamal, N. M., Mogahed, E. A., El-Husseini, M. A., & Aljabri, M. F.*** Perinatal transmission of hepatitis C virus: an update. Archives of Medical Science. 2019.

[29]. ***El-Zanaty F, Way A.*** Egypt demographic and health survey 2008. (Egyptian: Ministry of Health and Associates and Macro International, Cairo); 2009.

[30]. ***Enomoto A, Yoshino S, Hasegawa H, Komatsu T and Sasahara H.*** Phylogenetic investigation for the risk of hepatitis C virus transmission to surgical and dental patients. Journal Viral Hepat, 2001; 8(2): 148- 153.

[31]. ***Epstein RL, Sabharwal V, Wachman EM, et al.*** Perinatal Transmission of Hepatitis C Virus: Defining the Cascade of Care. J Pediatr. 2018; 203:34–40.e1.

[32]. ***Esmat G.*** Situation of HCV in Egypt Towards an End to HCV Epidemic, http://www.liver-eg.org/assets/esmatt-hepatitis-c-situationin-egypt.pdf 2014.

[33]. ***Faridulla Salih M, Malik IA, Hussain I.*** Increasing prevalence of chronic hepatitis and associated risk factors. Pak J Med Res. 2002; 41:46–50.

[34]. ***Garcia-Tejedor A, Maiques-Montensinos V, Diago-Almela VJ,et al.*** Risk factors for vertical transmission of hepatitis C virus: a single center experience with 710 HCV-infected mothers. Eur J Obstet Gynecol Reprod Biol 2015; 194:173–7.

[35]. ***Goyal LD, Kaur S, Jindal N, Kaur H.*** HCV and pregnancy: prevalence, risk factors, and pregnancy outcome in north Indian population: a case-control study. J Obstet Gynaecol India. 2014; 64(5):332–336.

[36]. ***Habib M, Mohamed MK, Abdel-Aziz F, Magder LS and Abdel-Hamid M.*** Hepatitis C virus infection in a community in the Nile Delta: risk factors for seropositivity. Hepatology, 2001; 33(1): 248-253.

[37]. ***Hanafiah M K, Groeger J, Flaxman AD, Wiersma ST.*** Global epidemiology of hepatitis C virus infection: new estimates of age-specific antibody to HCV seroprevalence. Hepatology 2013; 57: 1333-1342.

[38]. ***Huang Q, Hang L, Zhong M, Gao Y, Luo M, Yu Y.*** Maternal HCV infection is associated with intrauterine fetal growth disturbance. Medicine (Baltimore) 2016; 95:1-7.

[39]. ***Kalil KA, Farghally HS, Hassanein KM, Abd-Elsayed AA and Hassanein FE*** . Hepatitis C virus infection among paediatric patients attending University of Assiut Hospital, Egypt. East Mediterr Health Journal 2010; 16(4): 356-361.

[40]. ***Kamal SM, Nasser AI.*** Hepatitis C genotype 4: What we know and what we don't yet know. Hepatology. 2008; 47(4): 1371–1383.

[41]. ***Kandeel A, Genedy M, El-Refai S, Funk AL, Fontanet A, Talaat M.*** The prevalence of hepatitis C virus infection in Egypt 2015: implications for future policy on prevention and treatment. Liver Int.2017;37(1):45-53.

[42]. ***Karnsakul W, Schwarz KB.*** Management of hepatitis C infection in children in the era of direct-acting antiviral agents. J Viral Hepat. 2019;26(9):1034-1039.

[43]. ***Khamis, H. H., Farghaly, A. G., Shatat, H. Z., & El-Ghitany, E. M.*** Prevalence of hepatitis C virus infection among pregnant women in a rural district in Egypt. Tropical Doctor 2014; 46(1), 21–27.

[44]. ***Kim, D. et al.*** Cost-effectiveness model for hepatitis C screening and treatment: Implications for Egypt and other countries with high prevalence. Global public health 2015; 10, 296–317.

[45]. ***Kouyoumjian SP, Chemaitelly H, Abu-Raddad LJ.*** Characterizing hepatitis C virus epidemiology in Egypt: systematic reviews,

meta-analyses, and meta-regressions. Sci Rep. 2018; 8(1):1661.

[46]. ***Lankarani KB, Ardebili M, Sepehrimanesh M, Nejabat M, Hemmati Rad MA, Hosseini SY.*** Evaluation of hepatitis C virus intra-familial transmission among families with one index case, a pilot study from Fars province, Iran. Gastroenterol Hepatol Bed Bench. 2016; 9(4):250–258.

[47]. ***Lavanchy D.*** Evolving epidemiology of hepatitis C virus. Clin Microbiol Infect 2011; 107: 115.

[48]. ***Le Campion A, Larouche A, Fauteux-Daniel S, Soudeyns H.*** Pathogenesis of hepatitis C during pregnancy and childhood. Viruses 2012; 4:3531–3550.

[49]. ***McDermott, CD, Moravac, CC, Yudin, MH.*** The effectiveness of screening of Hepatitis C virus in pregnancy. JOGC 2010; 32: 1035–1041.

[50]. ***McNeil Jr., Donald G.*** Curing Hepatitis C, in an Experiment the Size of Egypt. The New York Times. 2015; December 16.

[51]. ***Ministry of Health and Population [Egypt], El-Zanaty and Associates [Egypt], & ICF International. (2015).*** Egypt Health Issues Survey 2015. Retrieved 9 December 2015, from http://dhsprogram.com/what-we-do/survey/survey-display-480.cfm

[52]. ***Mishra N, Mishra VN, Thakur P.*** Study of Abnormal Liver Function Test during Pregnancy in a Tertiary Care Hospital in Chhattisgarh. J Obstet Gynaecol India. 2016; 66(Suppl 1):129–135.

[53]. ***Nwaohiri A, Schillie S, Bulterys M, Kourtis AP***. Hepatitis C virus infection in children: how do we prevent it and how do we treat it? Expert Rev Anti Infect Ther. 2018; 16(9):689-694.

[54]. ***Omar MZ, Metwally MA, El-Feky HM, Ahmed IA, Ismail MA, Idris A.*** Role of intra-familial transmission in high prevalence of hepatitis C virus in Egypt. Hepat Med. 2017; 9:27–33.

[55]. ***Pergam SA, Wang CC, Gardella CM, Sandison TG, Phipps WT, Hawes SE.*** Pregnancy complications associated with hepatitis C: data from a 2003-2005 Washington state birth cohort. Am J Obstet Gynecol 2008; 199: 38.e1-38.e9

[56]. ***Prasad MR, Honegger JR.*** Hepatitis C virus in pregnancy. Am J Perinatol 2013; 30: 149-59.

[57]. ***Reddick KLB, Jhaveri R, Gandhi M, James AH, Swamy GK.*** Pregnancy outcomes associated with viral hepatitis. J Viral Hepat 2011; 18:e394-8.

[58]. ***Saleh DA, Shebl F, Abdel-Hamid M, Narooz S, Mikhail N, El-Batanony M, et al.*** Incidence and risk factors for hepatitis C infection in a cohort of women in rural Egypt. Trans R Soc Trop Med Hyg. 2008; 102(9):921-928.

[59]. ***Shebl FM, El-Kamary SS, Saleh DA, Abdel-Hamid M, ..et al.*** Prospective cohort study of mother-to-infant infection and clearance of hepatitis C in rural Egyptian villages. J Med Virol. 2009; 81: 1024-1031

[60]. ***Stoszek SK, Abdel-Hamid M, Narooz S, El Daly M, Saleh DA, Mikhail N, et al.*** Prevalence of and risk factors for hepatitis C in rural pregnant Egyptian women. Trans R Soc Trop Med Hyg. 2006; 100(2):102–107.

[61]. ***Tosone G, Maraolo AE, Mascolo S, Palmiero G, Tambaro O, Orlando R.*** Vertical hepatitis C virus transmission: Main questions and answers. World J Hepatol 2014; 6:538–548.

[62]. ***Tovo PA, Calitri C, Scolfaro C, Gabiano C, Garazzino S.*** Vertically acquired hepatitis C virus infection: Correlates of transmission and disease progression. World J Gastroenterol. 2016; 22:1382–1392.

[63]. ***World Health Organization.*** Global Health Sector Strategy on Viral Hepatitis, 2016–2021, (Geneva, Switzerland: World Health Organization, 2015).

[64]. ***Yeung LT, To T, King SM, Roberts EA***. Spontaneous clearance of childhood hepatitis C virus infection. J Viral Hepat. 2007;14(11):797-805.

[65]. ***Zahran, KM, Badary, MS, Agban, MN, Abdel Aziz, NH.*** Pattern of hepatitis virus infection among pregnant women and their newborns at the Women's Health Center of Assiut University, Upper Egypt. Int J Gynaecol Obstet 2010; 11: 171–174.